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Comparison of Urine and Blood Testing for HIV among Pregnant Women in Calabar, Nigeria

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Abstract
Testing for HIV is an essential component of the diagnosis and treatment of persons infected with the virus, and antenatal care provides a golden opportunity for detection of HIV infection in women of childbearing age. The use of blood sample to test for HIV has been the gold standard in clinical practice. However other body fluids such as urine, cervical secretion, tears, and saliva have potential as alternative media for HIV testing. This was a comparative noninferiority experimental study, comparing rapid diagnostic HIV testing using urine and blood samples. Two hundred and fifty new antenatal care clients and laboring women of unknown HIV status, were randomly recruited from antenatal care and delivery units. The seroprevalence of HIV was 6.31 and 5.86%, with use of urine and blood samples, respectively. The use of both urine and blood samples yielded a sensitivity of 100%. However, specificity was 99.05 and 99.52% for urine and blood samples, respectively. Area under receiver operating characteristic (ROC) curve was 0.995 and 0.998 for urine and blood samples, respectively. There was no significant difference in subjects' perception toward the use of blood or urine for HIV testing ($p > 0.05$). More subjects however preferred the use of blood rather than urine for HIV testing. In keeping with previous studies, the use of urine or blood for HIV testing in this study yielded similar results, with comparable sensitivities and specificities. Urine samples may therefore well be considered as alternative to blood samples for HIV testing. The use of urine should be considered by health-care providers as suitable alternative to blood for HIV testing. Pregnant women should be educated on the benefits and accuracy of using urine for HIV testing, for improvement in the perception toward its usage and its acceptability and preference.

Keywords: HIV; Urine testing; Blood testing; Sensitivity; Specificity; Pregnant women.

1. INTRODUCTION

Testing for HIV is an essential component of the diagnosis and treatment of persons infected with the virus, screening of blood for transfusion, and HIV/AIDS related research. Thus accurate and cost-effective testing is of great importance in combating the spread of HIV and in ensuring quick interventions for both the mother and baby where indicated. It is also imperative that tests for the diagnosis of HIV infection should be as accurate as possible given the serious ethical, legal, and social issues that accompany HIV infection [1-5].

Early identification and treatment of HIV infection in pregnant women not only improve the health of the mother but are the best ways to prevent neonatal disease [6, 7]. Testing in pregnancy allows detection of this group of women who would benefit from newer antiretroviral medications given to women with HIV during pregnancy and labor and to their newborns in the first hours after birth. The use of these antiretroviral drugs along with other interventions can reduce the rate of mother-to-child HIV transmission from 25% to less than 1%. Without treatment, approximately one in four exposed babies will be infected with HIV [7].

The use of body fluids other than blood such as saliva and urine as specimens for detecting antibodies to HIV has been reported to have potential as an alternative strategy for HIV testing [8, 9]. The use of urine samples may be attractive because of the ease of sample collection, cost-effectiveness, better safety (against needle injuries), and higher compliance rates [8, 9]. Testing of these types of specimen can be a useful alternative when it is difficult or impossible to test for HIV in blood samples. Taking a blood sample is invasive and requires possession of minimum skills by the personnel [9]. There is also a need to correctly handle and dispose of sharps, which may not be feasible in some locations [9]. At times, it may not be possible to draw blood for religious reasons or difficulties may be experienced in collecting blood samples in hard-to-reach places where it is, nevertheless, important to have epidemiological surveillance. An alternative sample would be useful in such situations [3].

Urine is often collected routinely for analysis for protein, sugar, and nitrites in pregnancy [10, 11]. Adding HIV testing into the panel of investigations that are carried out with urine is simple and cost effective and is less likely to meet with resistance from the women. The HIV urine test is expected to broaden the acceptance and availability of HIV testing worldwide. It offers several important advantages compared to blood-based HIV test [12-14]. These include greater safety and ease of use for health-care workers, lower cost of sample collection, and stronger consumer/client acceptance [12]. Urine sample collection is noninvasive and there is no need for expensive facilities and equipment or for highly trained personnel [13]. Urine antibodies retain activity under normal conditions of transport and storage and therefore appear to have widespread application [14].

1.1. Justification for the Study

The prevention of mother-to-child transmission (PMTCT) of HIV hinges on identification of pregnant women who are HIV positive in order to institute appropriate care and intervention. Testing for HIV using blood has been the standard of practice in our center, nationally, and globally. Drawing blood requires technical skills, is invasive, and carries the additional requirement of appropriate and safe disposal of sharps. Its use therefore limits running HIV test to skilled providers and to certain places. This hampers the universal detection of HIV positive individuals, including pregnant women. There is therefore a need for alternative testing strategy using other body fluids.

The use of urine sample to screen for evidence of diseases such as preeclampsia and asymptomatic bacteriuria is a routine practice in antenatal clinics (ANCs). Therefore, use of urine as an alternative medium for HIV testing is attractive because of the ease of collection, simplicity, better safety compared to blood, cost-effectiveness, noninvasive nature, and the fact that it can be performed by personnel with minimal skills. Furthermore, it can also be performed outside hospital settings such as traditional birth attendants' (TBA) homes, churches, medical outreaches, and homes and hence this increases the uptake of HIV screening and possible interventions to prevent mother-to-child transmission.

This study is therefore designed to determine the usefulness of urine HIV testing in identifying pregnant women with HIV infection at the ANC and labor ward of the University of Calabar Teaching Hospital (UCTH), Calabar. The findings from this study will help modify HIV testing strategy outside orthodox health-care facilities and ensure greater uptake of HIV counseling and testing.

2. METHODS

2.1. Study Location

The study was carried out at the ANC and labor ward of the UCTH, Calabar, Cross River State, Nigeria. The clinic runs on all working days, with an average weekly attendance of 500 clients. Women's views about using urine sample vis-à-vis blood for HIV test were enquired using structured questionnaire.

2.2. Study Design

This was an experimental study involving non-inferiority diagnostic test comparison.

2.3. Study Population

The study population comprised of ANC first attendees and unbooked labor ward clients with unknown HIV serostatus at the UCTH.

2.4. Sampling Method

Consecutive recruitment was carried out among all consenting pregnant women who presented at the ANC as well as unbooked pregnant women who were present at the labor ward. This was done until the required sample size was achieved.

2.5. Inclusion Criteria

1. Clients attending ANC at UCTH
2. Client delivering in labor ward at UCTH

2.6. Exclusion Criteria

1. Clients who did not give their consent
2. Clients who were unable to understand the explanation of the study, such as deaf-and-dumb clients and those with psychiatric problems
3. Unconscious clients, such as epileptics, and patients in diabetic coma and with head injuries

2.7. Ethical Consideration

Approval to carry out the study was obtained from the Hospital Ethical Committee before commencement.

2.8. Data Analysis

Data was collated and analyzed by an independent statistician using the Statistical Package for Social Sciences (SPSS) software, version 18. Categorical data was analyzed using the chi-square (x^2) and Fischer exact tests. Confidence interval and *p*-value were calculated. Level of significance was set at $p < 0.05$. A two-by-two table was used to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the urine test against the blood test.

3. RESULTS

3.1. Sociodemographic Characteristics

Two hundred and twenty-two pregnant women attending ANC at the UCTH, Calabar, within the three-month period of the study were recruited. Table 1 shows the sociodemographic characteristics of the subjects. The mean age was 28.1 (4.6) years

with a range of 17-40 years. Parity ranged from 0 to 5 with a mean of 0.86. The mean gestational age was 24 (7.3) weeks with a range of 8-39 weeks. About a third of the women were in their third trimester while more than half were in their second trimester.

Most of the women (90, or 40.5%) were in business; 54 (24.3%) were civil servants while only 2 (0.9%) were public servants.

The predominant religion was Pentecostal Christianity (193, or 86.9%) while only six (2.7%) were Muslims. The major ethnic groups represented were Ibibio (25.7%), Efiks (23.4), and Ibos (18.0%). One hundred one (45.5%) subjects had at least tertiary education while at least one-half (115, or 51.8%) had secondary education. Only six (2.7%) subjects had primary education.

Table 1: Sociodemographic characteristics of respondents.

Characteristics		Frequency	%
Age groups	<18	1	0.5
	18-25	63	28.4
	26-35	143	64.3
	>35	15	6.8
Gestational age	First trimester	17	7.7
	Second trimester	130	58.6
	Third trimester	75	33.7
Occupation	Civil servant	54	24.3
	Housewife	15	6.8
	Businesswoman	90	40.5
	Public servant	2	0.9
	Others	61	27.5
Denomination	Pentecostal	193	86.9
	Roman Catholic	12	5.4
	Jehovah's Witness	7	3.2
	Muslim	6	2.7
	Others	4	1.8
Ethnicity	Ibibio	57	25.7
	Efik	52	23.4
	Ibo	40	18.0
	Yakurr	7	3.2
	Others	66	29.7

Fourteen (6.3%) and thirteen (5.8%) of the 222 subjects tested positive for HIV with use of the urine and blood samples, respectively. All the subjects who were confirmed to be HIV positive also tested positive with use of both urine and blood samples, yielding a sensitivity of 100% for each of the testing methods. Also, 208 and 209 of the subjects tested negative for HIV with use of urine and blood samples, respectively. Two of the urine samples as well as one of the blood samples were falsely positive, yielding a specificity of 99.05 and 99.52%, respectively. There was no significant difference in sensitivity and specificity comparing the use of urine and blood samples for HIV testing ($p = 0.83$). Discordant testing result was found in only one of the subjects, who tested positive with use of urine sample and negative with use of blood sample, but the person was confirmed to be HIV negative. Also, one of the subjects who was positively concordant using urine and blood samples, was confirmed to be HIV negative with use of a tiebreaker. A PPV of 85.71% and an NPV of 100% was obtained with use of urine samples, while a PPV of 92.31% and an NPV of 100% was obtained with use of blood samples (See Tables 2-4).

Table 2: Diagnostic testing results for HIV screening using urine samples.

Diagnosis/Test	Confirmed HIV positive	Confirmed HIV negative	Subtotal
Test positive	12	2	14
Test negative	0	208	208
Subtotal	12	210	**222**

Table 3: Diagnostic testing results for HIV screening using blood samples.

Diagnosis/Test	Confirmed HIV positive	Confirmed HIV negative	Subtotal
Test positive	12	1	13
Test negative	0	209	209
Subtotal	12	210	**222**

Table 4: Comparison of HIV diagnostic testing using urine and blood samples (*N* = 222 pairs).

Diagnostic test characteristics	Urine sample (*N* = 222)	Blood sample (*N* = 222)
Positive result		
True positive (TP)	12	12
False positive (FP)	2	1
Negative result		
True negative (TN)	208	209
False negative (FN)	0	0
Seroprevalence (TP+FP)/N (%)	6.31	5.86
Sensitivity (%) (95% CI)	100 (75.75-100)	100 (75.75-100)
Specificity (%) (95% CI)	99.05 (97.73-100)	99.52 (98.59-100)
Area under ROC curve (95% CI)	0.995 (0.987-1.000)	0.998 (0.992-1.000)
Positive predictive value (%) (95% CI)	85.71 (67.38-100)	92.31 (77.82-100)
Negative predictive value (%)	100	100
Likelihood ratio (95% CI)	105.26 (26.4-417.1)	210 (29.7-1483.8)
Accuracy (TP+TN / TP+TN+FP+FN) (%)	99.10	99.55

3.2. Hypothesis Statement

Findings from this study indicate similarity in the sensitivity, specificity, positive and NPV as well as areas under the receiver operating characteristic (ROC) curve, with no statistically significant difference in these parameters, comparing the use of urine and blood for HIV testing. This study therefore fails to reject the null hypothesis (H_o) and states that a rapid diagnostic test for HIV infection in pregnant women using urine HIV test kits is as sensitive and accurate as the use of blood HIV test kits.

Two hundred and four respondents (91.9%) had previously tested for HIV before the study period at either less than 6 months (103 or 46.6%), 7-12 months (21, or 9.5%), 1-2 years (33, or 14.9%), 2-5 years (28, or 13.7%), or more than 5 years (16, 7.2%). About one-tenth (18, 8.1%) had never tested for HIV. Those who had never tested for HIV were significantly younger, with mean (SD) age of 23.2 (4.3) years, compared with those who had tested before, who had mean (SD) age of 28.52 (4.4) years ($t = 4.95$, $p = 0.000$). Those who had tested for HIV were also generally less parous with mean (SD) parity of 0.44 (0.8), compared with those who had tested before, who had mean (SD) parity of 0.9 (1.0), though this was not statistically significant ($t = 1.84$, $p = 0.07$). However, nontesting for HIV was significantly common among women who had not had any child yet (13, 12.4%), compared with those who had had at least one child (5, 4.3%) ($X = 4.9$, $p = 0.03$). Gestational age at presentation and other sociodemographic factors were not seen to be significantly associated with previous HIV testing status ($p < 0.05$).

Among those who had previously tested for HIV, the use of blood samples was much commoner (200, 98%) than the use of urine (4, 2.0%) ($X = 183$, $p < 0.01$). Table 3 shows the attitude of the women toward the use of urine and blood for HIV screening. Most of the respondents perceived the use of blood or urine samples to be good for HIV testing, with no significant

Figure 1: Receiver operating characteristics for sensitivity vs 1-specificity of HIV testing using blood and urine.

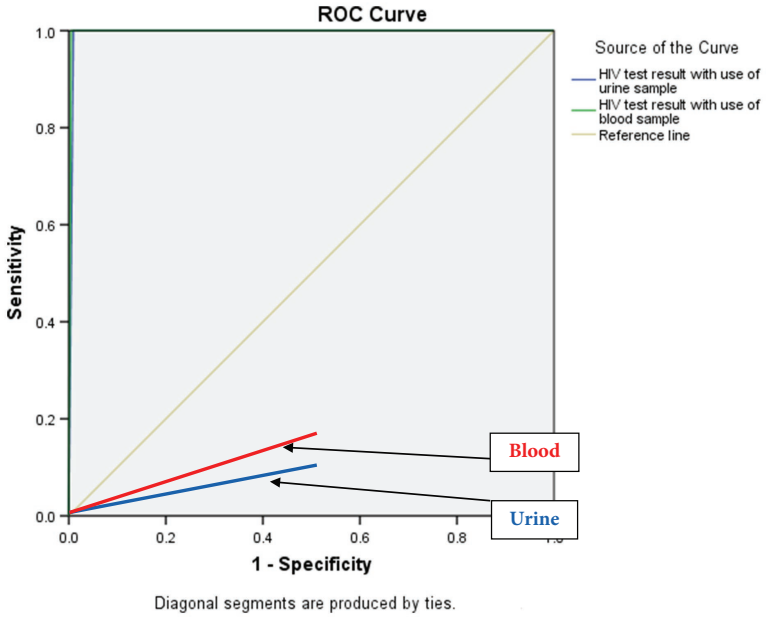

Figure 2: Likelihood ratio nomogram for HIV testing using urine and blood samples.

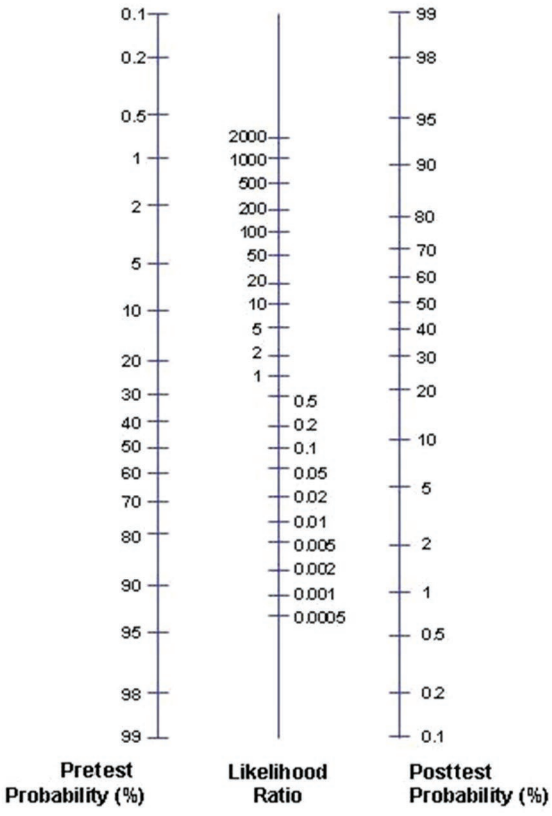

Table 5: Comparison of perception toward use of fluid samples for HIV testing (*N* = 222 pairs).

Characteristics	Urine sample *N* (%)	Blood sample *N* (%)	Total *N* (%)	Chi-square	*P*-value
Last HIV testing sample	4 (2.0)	200 (98)	204 (100)	188.3	0.000
Perception toward sample fluid					
Good	184 (48.8)	193 (51.2)	377 (100)		
Bad	4 (40.0)	6 (60.0)	10 (100)	2.74	0.254
Indifferent	34 (59.6)	23 (40.4)	57 (100)		
Preferred sample for HIV testing	98 (45.4)	118 (54.6)	216 (100)	1.85	0.174

difference seen in their perception toward use of urine or blood ($X = 2.74$, $p = 0.254$). However, the use of blood samples (98, or 45.4%) was preferred to the use of urine (118, or 54.6%) for HIV testing, though this difference was not statistically significant ($X = 1.85$, $p = 0.174$).

4. DISCUSSION

The aim of the study was to compare the use of urine and blood samples for rapid HIV-1/2 diagnosis as well as to assess the acceptability of using urine for HIV diagnostic testing among pregnant women.

The Alliance Biomedical Rapid HIV (1 and 2) urine test kit, which was used in this study, yielded a sensitivity and specificity of 100 and 99.05%, respectively, comparable to using the rapid blood diagnostic test, which also yielded a sensitivity of 100% but specificity of 99.52%. A similar study in Thailand using the urine rapid diagnostic test demonstrated a sensitivity of 99% and specificity of 100%.

Thus this study supports the findings of other investigators who posit that the use of urine samples for rapid HIV (1 and 2) testing is as sensitive and specific as the use of blood samples. Besides the similarity in their sensitivities and specificities, the use of urine and blood samples in this study yielded the same NPV of 100% and a similar PPV of 85.71% and 92.31%.

According to the WHO, an ideal test for the rapid diagnosis of HIV infection should be inexpensive, highly sensitive and specific, and easy to perform and interpret. In addition to these characteristics an ideal rapid test should be able to be stored at room temperature, should have long shelf lives, and should require no additional equipment or auxiliary supplies in order to be performed [6]. The urine-based rapid assay used in this study fulfills these criteria and as such serves as a powerful tool for HIV diagnosis.

The urine test provides an alternative to the use of blood, especially in situations where or when drawing of blood is impractical or unsafe or refused by patients. Many people, including pregnant women, have been noted to remain unaware of their HIV status, because of their fear of needles and resistance to the required bloodletting for HIV testing using blood samples. This may contribute significantly to the difficulty in controlling the spread of the HIV pandemic as well as uptake of the much-needed PMTCT interventions, especially in resource-poor settings.

Compared with the use of blood, the noninvasive use of urine for antibody-mediated detection of HIV 1 and 2 infection makes urine a safer alternative, as it virtually eliminates the potential risk of transmission of the virus to testing personnel. The collection of urine specimen is very flexible since it is a random void that does not require immediate refrigeration and could be used for both HIV testing and other routine analysis in pregnancy such as glucose and protein analysis.

The perception of the subjects toward their preferred sample fluid for HIV testing was also assessed, with about one-half of the subjects having good perception toward use of urine and blood. It is interesting to note that a large proportion of the subjects were however indifferent to which body fluid was used for testing.

Though more women preferred the use of blood to urine in HIV testing, the difference was not statistically significant. The lower preference for urine as a sample for HIV testing could be as a result of the low awareness of the availability of such testing medium. With more education and awareness, it is expected that the preference for urine will increase both for initial and for follow-up testing.

In this study, younger and less parous women have been identified to have low uptake of HIV testing, compared with older and more parous mothers. This underscores the need for improvement in reproductive health services especially among adolescent girls, including premarital, marital, and preconception counseling.

5. CONCLUSION

The use of urine samples for rapid diagnosis of HIV 1 and 2 is as sensitive and specific as using blood samples. Urine is also an acceptable source of body fluid for HIV testing in the study population. However, there is a need for improvement in pregnant

women's awareness of the use of urine samples for HIV diagnosis, as well as its utilization in the relevant maternal health service units.

5.1. Recommendations

1. The use of urine rather than blood for HIV testing, especially if urine samples are collected for urinalysis and other urine tests, is strongly recommended.
2. Further studies using larger samples in the study area and other regions within Nigeria are recommended as perceptions may differ in different parts of the country. These should include the perception of relevant health workers toward the use of urine for HIV testing.
3. For (pregnant) women with or without their spouses, health education on the utility and benefits of using urine for HIV testing should be instituted. This could be done at preconception and ANC counseling sessions.
4. Involvement of stakeholders and health policy makers toward support for use of urine for HIV testing is advocated. This should include support for sensitization of health workers and clients; development of manuals of the requisite health education; formulation, implementation, monitoring, and evaluation of enabling policies and research.
5. In view of the high prevalence of HIV infection in Nigeria, it is recommended that efforts should be made both by government and nongovernmental organizations to produce the test kits locally. This would make them cheaper and more accessible and generate employment opportunities.
6. Cost-effectiveness studies comparing the use of urine and blood samples for HIV testing, especially in resource-poor settings like ours, are recommended.

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Authors' Contributions

E.E. Omoronyia: Conception and design of study, literature search, organization of study, acquisition of data, drafting the article, revising it critically for important intellectual content, final approval of the article for publication.
M.I. Ekott: Conception and design, drafting the article, supervision of proposal, revising it critically for important intellectual content, final approval of the article for publication.
O.E. Omoronyia: Conception and design, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content, final approval of the article for publication.
A. Udo: Conception and design, revising it critically for important intellectual content, final approval of the article for publication.

Conflict of Interest

None.

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